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ASPECTS OF CELLULAR GROWTH AND DEVELOPMENT IN *NICOTIANA TABACUM* TISSUE CULTURED *IN VITRO*[*]

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ABSTRACT

The growth and development of long, multicellular, branched pseudothalli from tobacco tissue segments grown on nutrient agar in the presence of 4,0 mg/l indoleacetic acid and 0,08 mg/l kinetin, was investigated. Pseudothalli growing from the upper surface of an explant are 3—4 mm high and consist of tubular, bulbous and papillate cells. Their development follows a more or less sequential pattern along horizontal and vertical axes. On the undersurface pseudothalli are associated with giant cells and filaments growing on or into the agar. Following root formation and development, numerous root hairs, some dilated and bulbous, are established in the agar, between pseudothalli, or even in the air space above the callus.

UITTREKSEL

ASPEKTE VAN DIE SELLULÊRE GROEI EN ONTWIKKELING VAN *NICOTIANA TABACUM* WEEFSEL WAT *IN VITRO* GEKWEEK IS.

Die groei en ontwikkeling van lang, multisellulêre, vertakte pseudotalli vanuit tabakweefselsegmente wat op voedingsagar in die teenwoordigheid van 4,0 mg/l indoolasynsuur en 0,08 mg/l kinetien gekweek is, is ondersoek. Pseudotalli wat op die boonste oppervlak van 'n eksplant ontwikkel is 3—4 mm lank en bestaan uit buisagtige, bolvormige en papillêre selle. Hul ontwikkeling volg 'n min of meer sekwensiële patroon d.m.v. horisontale en vertikale vlakke. Op die onderkant van die eksplant is die pseudotalli met reusagtige selle en filamente wat òf op die agar òf net onderkant die oppervlakte van die agar groei, geassosieer. Na wortelvorming ontwikkel menigvuldige wortelhare, sommige waarvan verrek en bolvormig is, in die agar, tussen die pseudotalli, of selfs in die lugruim bokant die eksplant.

INTRODUCTION

Gautheret (1966) has enumerated in detail on the various cellular types that are susceptible to dedifferentiation. We find it interesting, too, that whereas companion and parenchyma cells of the intraxylary phloem and xylem parenchyma of the medullary rays generally show a greater tendency to dedifferentiate than the pith parenchyma of tobacco stem tissue, the result of dedifferentiation does not depend on the nature of the initial tissue. In tobacco, namely, the newly-formed callus produced by xylem parenchyma is the same as that produced by the parenchyma of the internal phloem bundles (Ellis and Bornman, 1970).

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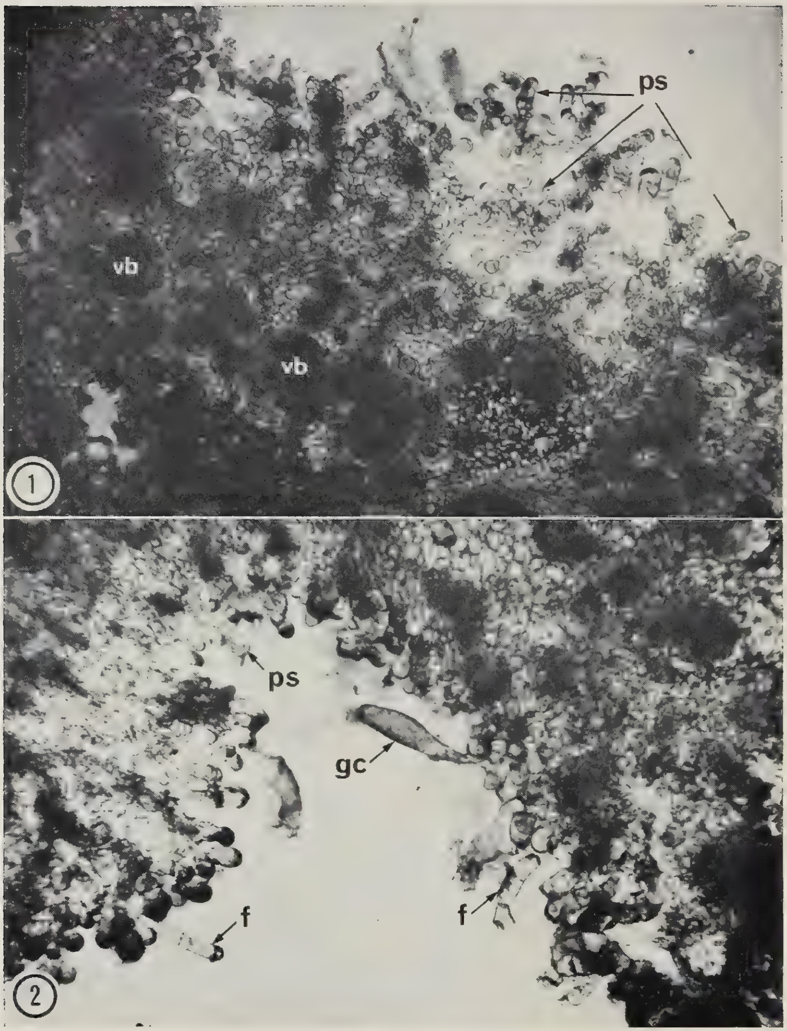


FIGS. 1–2.

Fresh tissue, polychrome stain, X36.—FIG. 1. Pseudothalli and vascular bundles on upper surface of explant.—FIG. 2. Loose, irregular callus on under-surface of explant.
f, filament; gc, giant cell; ps, pseudothalli; vb, vascular bundle.

It is commonly observed that a crystalline-like mass of callus becomes established from tobacco stem segments grown in the presence of levels of high auxin and low cytokinin and it appeared instructive to examine, in turn, those cell types which differentiate in response to the initial dedifferentiation of cells in the explant. Closer examination of such an explant's surface growth shows that it is made up of individual chains of cells, loosely packed together, giving the callus some resilience and a snowy to greenish-white appearance. The cells making up the chain appear to be in various stages of differentiation.

MATERIALS AND METHODS

The conditions under which stem explants of *Nicotiana tabacum* cv. Kutsaga 614 were grown, were reported on in detail in a previous paper (Ellis and Bornman, 1970). All observations and measurements were made on fresh material, unstained or stained with aniline blue, polychrome, or safranin, or viewed under phase contrast. Since a feature of particular interest was the development of pseudothalli, this study was restricted to callus cultured on a basal medium in the presence of 4,0 mg/l indoleacetic acid (IAA) and 0,08 mg/l kinetin (CK).

RESULTS AND DISCUSSION

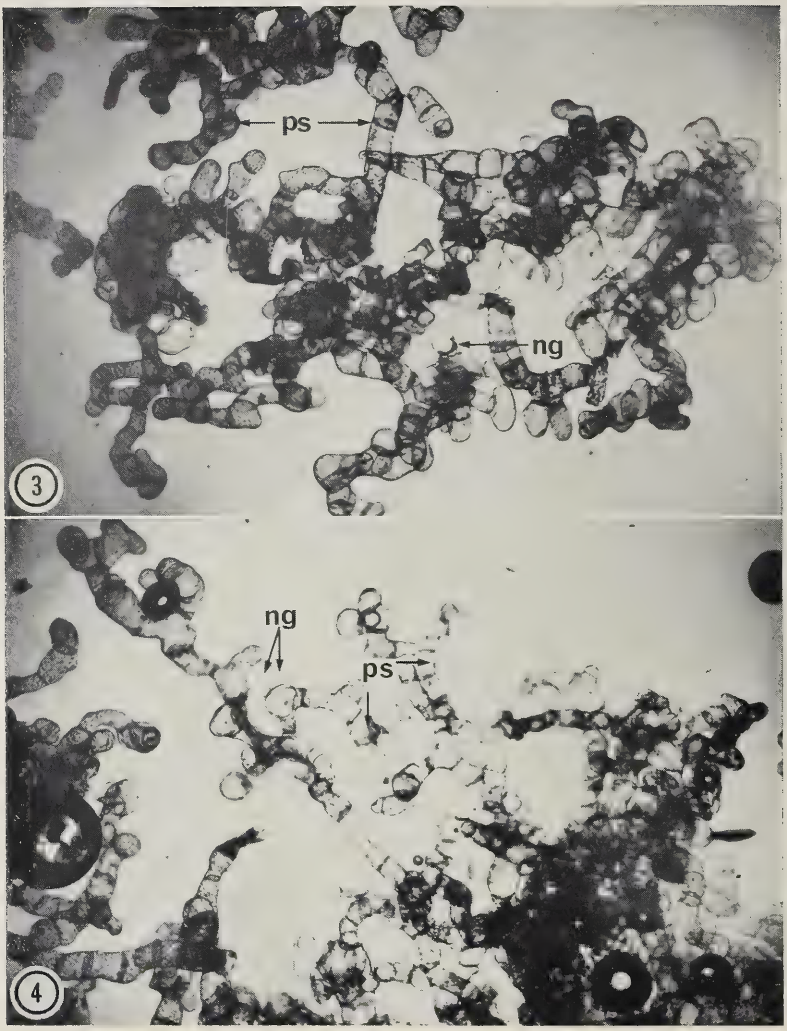
Characteristic of tobacco tissue cultured on nutrient agar in the presence of a high level of auxin, e.g. 4,0 mg/l IAA, is the development after four to five weeks of a mass of branched cellular filaments, the so-called pseudothalli of Gautheret (1957), giving it a downy or frosty appearance. There appears to be a distinct difference between supra-explant or aerial (Fig. 1) and sub-explant (Fig. 2) pseudothallial development.

Aerial pseudothalli

Pseudothalli developing on the upper surface of the stem explant into the air space above the agar, consist of multicellular, multibranched, rather loosely-knit chains (Figs. 3, 4). At their bases the pseudothalli are intergrown and dense, but toward their apices they are only loosely interconnected, if at all. In fact, most of the cells of a pseudothallus divide, grow, and develop in free air space. The individual filaments consist of approximately 20—30 and sometimes up to 40 cells, probably the upper limit in terms of osmotic absorption, and are from 3—4 mm high. Basically, pseudothalli are composed of four types of cells: tubular, bulbous or pear-shaped, papillate, and tracheary. The tracheary cells are discussed in a subsequent paper.

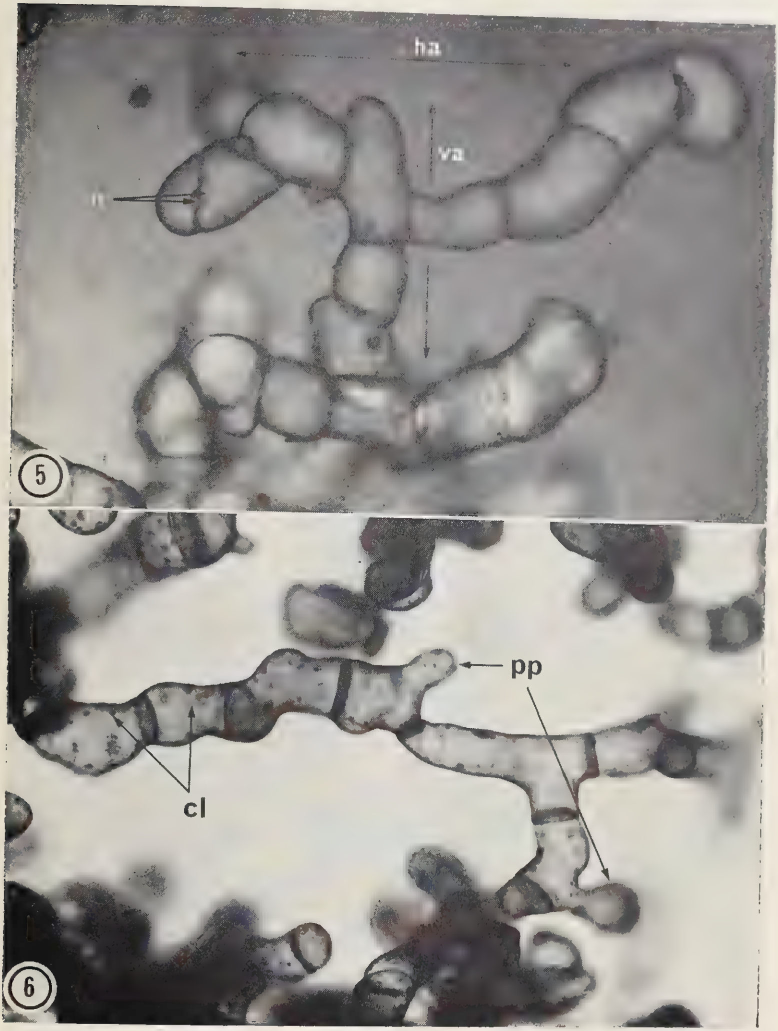
Tubular cells

The main body of the pseudothallus consists of long (up to 200 um) tubular cells, often with bulging protuberances. The younger division products are



FIGS. 3–4.

Fresh tissue, safranin, X60.—FIG. 3. Pseudothallial cells with numerous chloroplasts.—FIG. 4. Newly-budded cells devoid of chloroplasts.
ng, newly-budded cells; ps, pseudothalli.



FIGS. 5–6.

Fresh tissue, aniline blue stain, X160.—FIG. 5. Pseudothallus showing development of vertical and horizontal axes.—FIG. 6. Pseudothallus showing protrusion divisions which ultimately will form columns of cells at right angles. Papillae clearly visible. cl, chloroplast; ha, horizontal axis; va, vertical axis; n, nucleus; pp, papilla.

densely protoplasmic, exhibit active cyclosis, and have abundant mitochondria and undifferentiated plastids. The older cells, particularly those at the base, become increasingly vacuolate but contain numerous chloroplasts. The cell walls are thin and are speckled with pit-field-like depressions. The tubular cells display some anomalous features of division and growth, and a remarkable resemblance was found in respect of types of behaviour between these cells and those in free suspensions studied by Steward, Mapes and Smith (1958).

Stimulated by the plant hormones cytokinin and auxin, some cells on the surface of the original explant first divide to form a horizontal row of cells (Fig. 5). At a point along this axis, as a result either of periclinal division or of budding, vertical growth commences (Fig. 6) and after repeated divisions parallel to the short diameter of the cells, a vertical column of tubular cells is established constituting the main body of the developing pseudothallus. At some point on this vertical axis lateral development again occurs as a result either of anticlinal division or of budding. Thus, with the establishment of alternating horizontal and vertical axes, and also by inclined cell division (Fig. 9), S-, T- and Y-shaped—and multibranched variations of these—pseudothalli are formed. Not infrequently, the characteristic winglike appearance in a suspension culture (Steward *et al.*, 1958) of a mother cell flanked by two daughter cells, also is seen in aerial cellular development (Fig. 4).

Bulbous cells

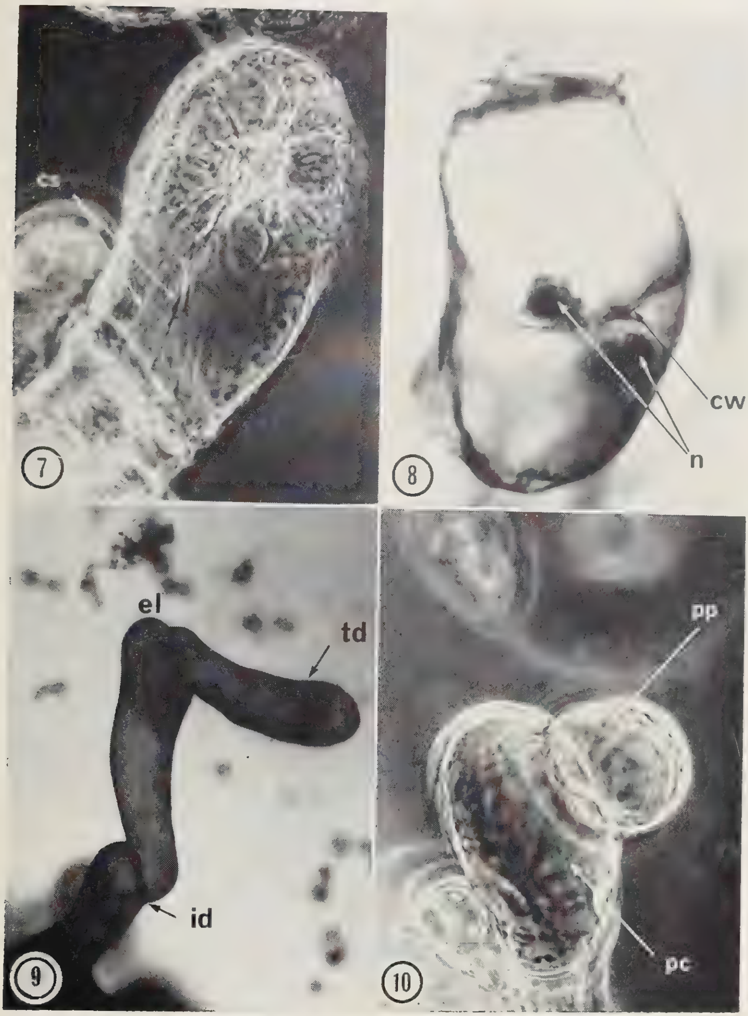
Terminal cells on the pseudothalli invariably are bulb, club or pear-shaped (Figs. 9, 10, 13, 14). Usually they are devoid of chloroplasts, hence the snowy appearance of such a culture colony, but the nuclei are large and protoplasmic streaming is active.

Papillate cells

On both tubular and terminal cells small protuberances or papillae often appear. These gradually enlarge although, initially at least, they remain constricted at the base (Figs. 6, 7). Papillae most likely are formed in response to a mitosis in the parent cell, part of whose increased volume of cytoplasm gradually pushes out into a bulge formed in a weakened area of the cell wall, probably a pit-field-like depression. Initially the papilla is a small, round cell, but once a mitotic nucleus has migrated into it, it enlarges, divides, and becomes tubular or, if in a terminal position, remains bulbous.

Sub-explant pseudothalli

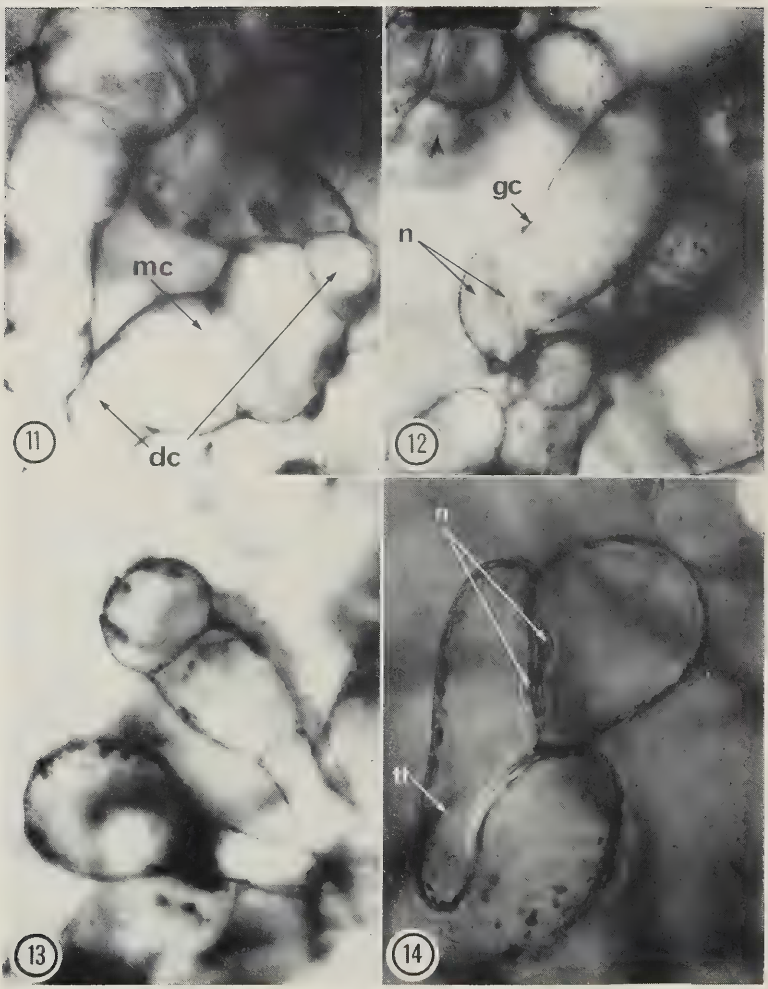
Pseudothalli on the undersurface of the stem explant are composed of short, dense cellular chains growing on, or slightly penetrating the agar surface. A feature of the sub-explant growth is the presence of giant cells and short filaments.



Figs. 7–10.

Fig. 7. Phase contrast view of cell from superficial zone of parenchyma showing nucleus suspended by cytoplasmic strands, X45.—Fig. 8. Transverse division in a surface cell, aniline blue, X500.—Fig. 9. Pseudothallus showing transverse and inclined division as well as an elbow-like structure, polychrome stain, X160.—Fig. 10. Papillus developing from parent cell, phase contrast, X400.

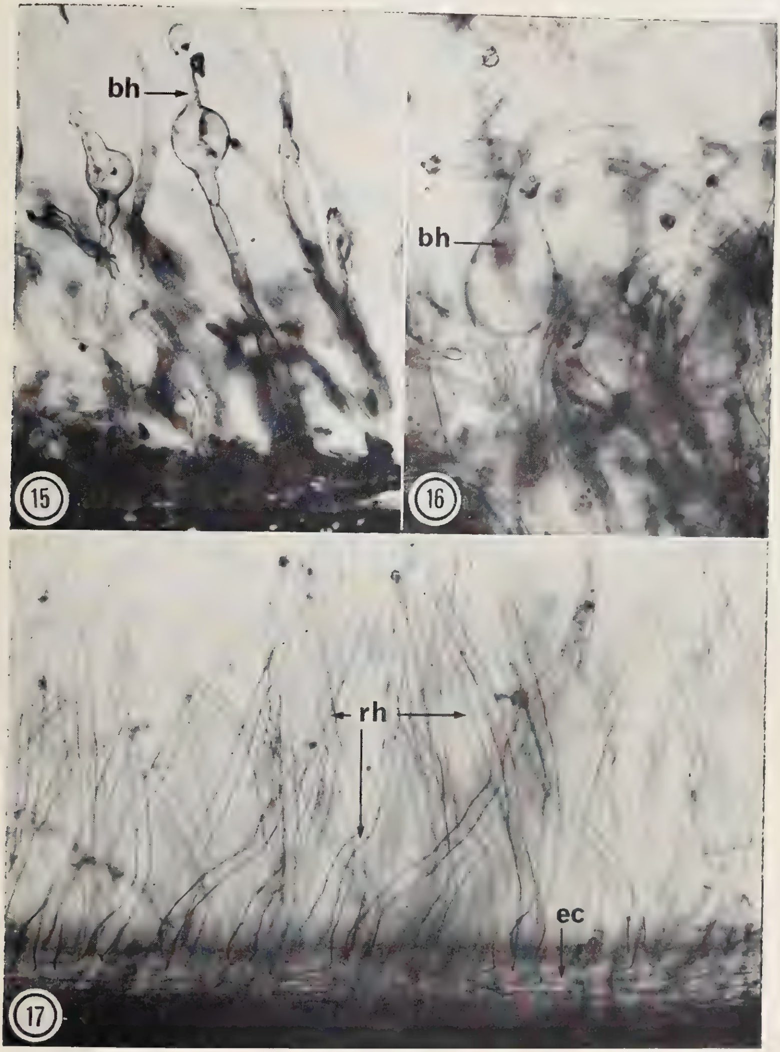
cs, cytoplasmic strands; cw, cell wall; el, elbow-like joint; id, inclined division; n, nucleus; pc, parent cell; pp, papillus; td, transverse division.



Figs. 11–14.

Fresh tissue, aniline blue stain.—Fig. 11. Wing-like structures resulting from successive transverse divisions on opposite sides of mother cell, X160.—Fig. 12. Irregular cell divisions (arrows) of a giant cell, X140.—Fig. 13. Terminal transverse divisions of pseudothalli, X160.—Fig. 14. Recently divided cells in superficial parenchyma showing a tail-like extension often characteristic of a developing tracheid, X350.

dc, daughter cell; gc, giant cell; mc, mother cell; n, nucleus; tt, tail-like extension.



Figs. 15–17.

Fresh mounts, aniline blue stain, X160.—Fig. 15. Root hairs with bulbous dilations growing in agar.—Fig. 16. Balloon-tipped root hair amongst pseudothalli.—Fig. 17. Aerial root hairs showing emergence from almost all epidermal cells.
ec, epidermal cells; bh, bulbous hair; rh, root hairs.

Giant cells

These are unusually large ($300\mu \times 100$—150 um), uninucleate cells (Figs. 2, 7, 8) with conspicuous cytoplasmic strands and are found only on the undersurface growing just above or in the agar. Giant cells appear to develop from the same type of surface cell which ultimately gives rise to a pseudothallus. Although highly vacuolated, giant cells divide, often forming daughter cells of unequal size. These divisions may continue until six or eight smaller cells occupy the original lumen of the giant cell. Steward *et al.* (1958) are of the opinion that the giant cells become polynucleate before internal divisions occur. The polynucleate conditions was not observed in the giant cells of our tobacco callus cultures.

Filaments

In freely suspended cell cultures unusually long, non-septate filamentous cells are often encountered. These have not be observed in tobacco tissue colonies but some cells, growing in close association with—or probably derived from—the giant cells, closely resemble them. Whether these filaments simply are the products of extension growth of the giant cells following wall formations in the latter, is not clear as yet. At least some filamentous growth can be traced back to large basal cells which are attached to giant cells and other surface cells by tail-like extensions (Fig. 14). Filaments are rarely composed of more than eight cells (Fig. 2) but could, as a result of division in various planes, probably give rise to branched pseudothalli.

Root hairs

With the attainment of equilibrium in pseudothallial development the larger proportion of growth in mass of the callus results from diffuse cambia which form immediately below the loose, irregular surface layers. Although isolated tracheary elements and islets of vascular tissue can be formed at the base of the surface pseudothallial layer, histogenic differentiation commonly occurs from the inner cells of the callus mass. Root primordia form and the developing roots course their way through the pseudothalli and grow into the nutrient agar or, not infrequently, exhibit negative geotropism and grow into the air space before curving back into the agar. Whether the root hairs develop in the agar (Fig. 15), in between the pseudothalli (Fig. 16), or in the air (Fig. 17), they are exceedingly numerous, each epidermal cell probably producing one. In the agar as well as in amongst the pseudothalli root hairs, up to 3 mm long, often exhibit huge, bulbous dilations.

CONCLUSION

The pseudothalli which develop in tobacco tissue in response particularly to a high auxin regime, consist of a number of different cell types in various phases of maturation. Their development from surface cells which, in turn, are the products of dedifferentiation of xylem ray and intraxylary phloem parenchyma (Ellis and Bornman, 1970), appear to follow a regular and repetable sequence of stages. The final form is probably governed to a large-degree by the ratio and/or concentrations of the plant hormones auxin and cytokinin.

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